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Methodological considerations in discriminating olive-orchard management type using olive-canopy arthropod fauna at the level of order

Carlos Jerez-Valle¹, Pedro A. García-López², Mercedes Campos³ and Felipe Pascual¹

¹ Universidad de Granada, Departamento de Zoología. Campus de Fuentenueva s/n, 18071 Granada, Spain. ² Universidad de Granada, Departamento de Estadística e Investigación Operativa. Campus de Fuentenueva s/n, 18071 Granada, Spain. ³ EEZ (CSIC), Departamento de Protección Ambiental. Profesor Albareda 1, 18008 Granada, Spain.

Abstract

The cultivation of the olive tree (*Olea europaea* L.) has great importance in the entire Mediterranean basin, so that the implementation of organic practices in their management directly affects the sustainability of the agricultural system. Bioindication with arthropods can help to detect the different agricultural practices. In this work, we analyse the most appropriate methodology for discriminating between management using arthropods at the taxonomic level of order, with the novelty of taking into account the weather conditions to select the sampling dates. Between 12 and 15 sampling stations (depending on the year) were selected from olive orchards belonging to organic, conventional non-tillage, and strict conventional management, being sampled by beating the canopy fortnightly in the spring-summer period of 2007, 2008 and 2009. Organic management was more abundant and richer than the rest for the three years. Most groups with significant differences in terms of relative abundance were more abundant in organic orchard, except Neuroptera. Finally, different discriminant methods were evaluated (Linear Discriminant Analysis, Multiple Discriminant Analysis, and Support Vector Machine) with several different data sets. The discriminant analysis with interannual variability reached 97.9% accuracy in differentiating between organic and non-organic management using the LDA method, considering the taxa with significant differences from the abundance, excluding pests, and using samples with more uniform and stable weather patterns (late summer).

Additional key words: bioindication; conventional; discriminant analysis; meteorological conditions; non-tillage; organic.

Abbreviations used: CON (conventional production); FR% (percentage of individuals recorded); K-W (Kruskal-Wallis non-parametric test); LDA (Linear Discriminant Analysis); m (slope of the line); MDA (Multiple Discriminant Analysis); *Me* (median); n (number of sample units used); $n_{(m=0.1)}$ (number of samples required for a slope of 0.1); N (absolute abundance of arthropods); NonORG (non-organic production); NS (not significant); NT (conventional non-tillage production); ORG (organic production); *Q1* (first quartile); *Q3* (third quartile); SD (standard deviation); *S*_{obs} (observed number of orders); SVM (Support Vector Machine); TNO (total number of orders); \bar{X}_{ORG} , \bar{X}_{NT} or \bar{X}_{CON} (mean abundance per station of each management type).

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Correspondence should be addressed to Carlos Jerez-Valle: cjerez@ugr.es.

Introduction

The general increase in the European agricultural landscape homogeneity during the second half of the 20th century has had a profoundly negative impact on biodiversity (Robinson & Sutherland, 2002; Benton *et al.*, 2003). The exacerbation of most of the problems

associated with insect pests has been associated with an expansion of monoculture at the expense of natural vegetation, reducing local habitat diversity and thereby seriously affecting the abundance and efficiency of natural enemies (Altieri & Letourneau, 1982).

Organic farming offers significant benefits for biodiversity, since it can potentially help balance large-

scale actions applied to the field (regulations and international agreements, agricultural policy, etc.) and local measures for specific areas designed to maximize the beneficial effects in habitats of interest and priority species (Hole *et al.*, 2005). Thus, to improve the functional biodiversity of agroecosystems is the key ecological strategy for achieving production sustainability (Altieri, 1999).

The cultivation of the olive tree (*Olea europaea* L.) is widespread throughout the Mediterranean region, especially in southern Spain. Currently, this crop occupies the largest surface area in Andalusia (Alonso, 2011). The natural and semi-natural vegetation in this region continues to be eliminated to increase the area for olive-orchard cultivation, and therefore the original landscape has been reduced and fragmented (Parra & Calatrava, 2006; Milgroom *et al.*, 2007). This trend has impoverished the arthropod fauna in the olive-orchard agroecosystem (Guzmán & Alonso, 2004b; Ruano *et al.*, 2004; Santos *et al.*, 2007a; Scalercio *et al.*, 2012; Paredes *et al.*, 2013). The surface area of organic olive orchards in Andalusia has almost doubled in the last 10 years (MAGRAMA, 2002, 2011), from 31,517 ha in 2002 to 56,023 ha in 2011. Today, conservation of olive production is a necessity for the fragile Mediterranean ecosystems and a challenge to all sectors involved (Loumou & Giourga, 2003).

Bioindication is a highly useful tool in this sense, as it permits the evaluation of the state of conservation of an ecosystem based on the living organisms that it contains (Büchs, 2003; McGeoch, 2007). The use of bioindicators for information on certain environmental variables have a long tradition (van Straalen & Verhoef, 1997). Arthropods have been widely used, especially in agricultural systems because they meet the requirements quite well of a good bioindicator, being: widely distributed, permanent residents, relatively abundant, easy to sample and identify, and vulnerable to pesticides. Most studies in this sense have used very low taxonomic levels (genus or species) from such families as Carabidae (Holland & Luff, 2000), Coccinellidae (Zahoor *et al.*, 2003; Cotes *et al.*, 2009) or Formicidae (Redolfi *et al.*, 1999, 2004; Andersen *et al.*, 2002; Pereira *et al.*, 2004) and orders such as Oribatida (Behan-Pelletier, 1999), Heteroptera (Fauvel, 1999) or Araneae (Marc *et al.*, 1999). Moreover, some studies suggest the use of higher taxonomic levels such as the order or family (Biaggini *et al.*, 2007; Scalercio *et al.*, 2009; Cotes *et al.*, 2011; Jerez-Valle *et al.*, 2014).

The main novelty of this work is that we consider the meteorological conditions during the dates of sampling in order to select the data set. Regardless of the taxonomic level considered, none of the previously mentioned authors evaluated weather conditions near

the sampling dates with this purpose. We propose the use of olive-orchard-canopy arthropod fauna at the taxonomic level of order to discriminate between different management types, including interannual variability and certain meteorological considerations about sampling dates, to answer the following questions:

a) Can the study of the high taxonomic levels canopy arthropod fauna of olive orchards discriminate the different orchard-management types? It is assumed that the use of high levels taxonomic arthropod fauna may be a useful tool for discriminating between management types, especially organic and non-organic.

b) What methodology is recommended to identify the management type, including the interannual variation? We hypothesise that taxa which maintain differences between management types throughout the years will be those that best discriminate between them, regardless of the method used.

c) What is the best period for sampling? We evaluated different sampling dates taking into account meteorological considerations to determine at what point the greatest differences occur between management types.

Material and methods

Study area and crop management systems

The study was conducted in the area of Montes Orientales, 30 km north of the city of Granada (Spain), for being one of the areas most intensely dedicated to traditional olive-orchard cultivation in the province of Granada (Guzmán & Alonso, 2004a). The study area occupies some 20 km from east to west and some 18 km north to south. This is a rather homogeneous zone, with altitudes of 960 to 1130 m asl. The climate is extreme continental Mediterranean, with long, cold winters, and equally long, warm summers. The mean precipitation does not reach 600 mm annually, rain falling mostly in the months of October to May. The main crop is olive, predominantly the cultivar 'Picual', grown under conventional as well as intensive systems. Also, cereals and other herbaceous crops abound in the area.

We considered three types of management when selecting the sampling stations, seeking uniformity regarding geographical and geophysical characteristics of the different olive-orchards.

Olive orchards under organic production (ORG hereafter), following EC Regulation no. 2092/91 of 24 June 1991, used environmentally friendly agricultural practices based on environmental and economic sustainability as well as self-regulation of the trophic

chains present in the agroecosystem. In this sense, no agrochemicals were used nor was the soil ploughed. During the study, only one treatment of *Bacillus thuringiensis* Berl. was applied between May and June, depending on the orchard.

In the olive orchards under conventional management, pesticide and herbicide use was widespread and routine, though two types of management were distinguished according to ploughing depth: strict conventional (CON hereafter), and conventional non-tillage (NT hereafter). In the latter, at most, shallow tillage was applied under the tree canopy at the beginning of spring. The agricultural characteristics of the different management types are listed in Table 1.

Experimental design and arthropod collection

Sampling was conducted for three consecutive years (2007, 2008, and 2009), between May and August, depending on the year, as the time when the largest differences are observed between management types (Redolfi *et al.*, 1999) and which has the highest arthropod abundance (Ruano *et al.*, 2004; Santos *et al.*, 2007a; Jerez-Valle *et al.*, 2014). The number of samples was reduced progressively from one year to the next, in the light of the results of accumulation curves for each campaign. In 2007, we selected 15 sampling stations, 5 for each type of management and sampled fortnightly on five dates between early June and early August. In 2008, we selected 12 sampling stations, 4 for each management type, taking samples every two weeks from May to July on four different dates. Finally, in 2009, we also selected 12 stations, sampling fortnightly but only on three different dates, between late May and June.

Each sampling station was composed of a row of four trees sampled, leaving one alternately unsampled. In this way, the distance between sampled trees was greater than 15 m, to ensure the independence of the data. In addition, the minimum distance between each station was 500 m.

We sampled the canopy by the beat-down technique (Wilson, 1962) for 40 seconds, beating four branches per tree (one per orientation) taken at random. We used a 45-cm-diameter sweep net with a rod 55 cm in length.

The content of the net (methodological sample) was emptied into plastic bags properly labelled, with a few drops of ethyl acetate as an anaesthetic to avoid predation. In the laboratory, all samples were stored in a freezer at -20°C until processing. The sampling unit was considered to be the average of four methodological samples taken at each station per sampling date, to avoid dispersion of data and increase uniformity.

Each sample was processed in the laboratory, separating and classifying the arthropods. Under a binocular microscope, all the adult and juvenile specimens were classified to the level of order, except in the case of *Euphyllura olivina* (Costa) (Hemiptera: *Psyllidae*) and *Prays oleae* (Bern.) (Lepidoptera: *Plutellidae*), which, for being rampant pests were considered apart. The family Formicidae was also separated from the rest of Hymenoptera due its high number. In addition, within the order Hemiptera, the suborder Heteroptera was separated from the suborders Auchenorrhyncha and Sternorrhyncha, which were lumped under the name “Other Hemiptera”.

Statistical analysis

Summary statistics. Absolute abundance of arthropods (N) was considered as the number of individuals recorded in total per management type. In addition, for each management type, the mean number of individuals per sampling station (\bar{X}) and standard deviation (SD) were calculated, differentiating samples taken on different dates by leaving at least 15 days between samplings in all cases. The median (*Me*) for each order and management type per year was also calculated as measure of central tendency, as well as the first and third quartiles (*Q1* and *Q3*, respectively).

Due to the non-normality of the data, despite several transformations, the Kruskal-Wallis non-parametric test (K-W) was used in addition to the Mann-Whitney U test, for multiple and two independent samples, respectively, to detect differences at 95% significance level. We also performed a pairwise comparisons analysis (Nikolić, 2007) to determine between which values such differences occurred. The calculations were made using R software (R Development Core Team, 2012).

Table 1. Summary of the agricultural characteristics of each type of management

Management	Abbreviation	Weed control	Pest control	Fertilizer	Tillage	Tree age (years)
Organic	ORG	Mowing	<i>Bt</i> [†]	Organic	No	20-25
Non-tillage	NT	Without mowing	Insecticides	Mineral	No	20-25
Conventional	CON	Herbicides	Insecticides	Mineral	Yes	15

[†] Treatment with *Bacillus thuringiensis*.

Accumulation curves. For each type of management, species-accumulation curves were constructed following the methodology of Jiménez & Hortal (2003). In this case, the curves refer to orders. Hemiptera was considered as two groups (Heteroptera and “Other Hemiptera”). The family *Formicidae* was included in Hymenoptera, while *E. olivina* in “Other Hemiptera” and *P. oleae* in Lepidoptera. The Clench fit was applied to each curve, by nonlinear estimation by the Simplex & Quasi-Newton method (StatSoft, 2005). To construct the accumulation curves from the real data, the program EstimateS (Colwell, 2009) was used, and the model was fit using the program Statistica 7.1 (StatSoft, 2005).

Meteorological data. Data from Agroclimatic Information Network of Andalusia (<http://www.juntadeandalucia.es/agriculturaypesca/ifapa/web>) and Crop Disease Warning Information Network (<http://www.juntadeandalucia.es/agriculturaypesca/raif/>) of the Junta de Andalucía were used to determine weather conditions on sampling days. We considered the stations located within 50 km from the centre of the study area (16 stations in total), recording the daily values of precipitation (mm), average temperature (°C), relative humidity (%) and daily radiation accumulated (MJ/m²). We performed a hierarchical cluster analysis using between-groups linkage method using the squared Euclidean distance, with data from each sampling date and for the previous four days. This provided a dendrogram classification with the results of the cluster. The calculations were performed using the R software (R, 2012).

Discriminant analyses. We built two discriminant functions to classify the olive-orchard stations, paying attention to the management type and considering the criterion “organic/non-organic”. Moreover, we used three different discriminant methods to determine the most efficient: Linear Discriminant Analysis (LDA) (Venables & Ripley, 2002; Ripley, 2008), Multiple Discriminant Analysis (MDA) (Hastie *et al.*, 1994, 1995; Hastie & Tibshirani, 1996) and Support Vector Machine (SVM) (Fan *et al.*, 2005; Chang & Lin, 2011).

In addition, two different analyses were developed based on the criteria for choosing the groups to include: analysis 1 (with all groups) and analysis 2 (with taxa that showed significant differences in the three years). Finally, looking for an improvement in classification rates, we considered meteorological criteria to divide the data set into two subsets of dates, which we analysed. All calculations were performed using the software R (R, 2012) with “package MASS” (Venables & Ripley, 2002), “package mda” (Hastie *et al.*, 2011) and “package e1071” (Dimitriadou *et al.*, 2011).

Results and discussion

Summary statistics and arthropod community

A total of 42,369 specimens were collected, belonging to 14 different orders (Heteroptera considered separately from other Hemiptera). In 2007, 25,602 specimens were collected, 11,275 in 2008 and in 2009, the highest abundance being of ORG (32,160 individuals within three years), followed by CON and NT (6,098 and 4,111, respectively).

By years, in 2007 the highest abundance was recorded in ORG with 22,990 individuals, followed by CON and NT (1,346 and 1,266 individuals, respectively). Significant differences were detected between ORG, NT, and CON management (Fig. 1a) with respect to medians ($Me_{ORG}=207.8$; $Me_{NT}=12.0$; $Me_{CON}=12.0$; K-W=49.5, $p<0.05$; mean abundance per station of each management type: $\bar{X}_{ORG}=249.9 \pm 18.3$; $\bar{X}_{NT}=13.1 \pm 0.8$; $\bar{X}_{CON}=13.6 \pm 0.9$). The analysis was repeated removing pests of great abundance (Fig. 1b), confirming the differences found in the previous analysis ($Me_{ORG}=14.0$; $Me_{NT}=8.8$; $Me_{CON}=5.8$; K-W=26.1, $p<0.05$; mean abundance per station: $\bar{X}_{ORG}=22.8 \pm 1.7$; $\bar{X}_{NT}=11.3 \pm 0.7$; $\bar{X}_{CON}=7.2 \pm 0.5$).

In 2008, abundance also proved highest in ORG with 6,879 specimens, followed by CON and NT (2,921 and 1475 individuals, respectively). Significant differences were detected, in this case between the three management types (Fig. 1c) with respect to the medians per station ($Me_{ORG}=122.3$; $Me_{NT}=19.1$; $Me_{CON}=44.0$; K-W=40.8, $p<0.05$; mean abundance per station: $\bar{X}_{ORG}=125.2 \pm 15.2$; $\bar{X}_{NT}=23.0 \pm 2.6$; $\bar{X}_{CON}=45.6 \pm 3.9$). Repeating the analysis without pests (Fig. 1d), we detected differences between ORG and the other two management types ($Me_{ORG}=14.8$; $Me_{NT}=10.0$; $Me_{CON}=10.9$; K-W=9.3, $p<0.05$; mean abundance per station: $\bar{X}_{ORG}=22.8 \pm 3.3$; $\bar{X}_{NT}=11.3 \pm 0.8$; $\bar{X}_{CON}=9.2 \pm 0.4$).

Finally, 2,291 individuals were collected in 2009 in ORG, this again being the management type with the highest abundance, followed by CON and NT (1,831 and 1,370, respectively). On this occasion, the only significant differences were between ORG and NT when considering the medians (Fig. 1e) ($Me_{ORG}=47.3$; $Me_{NT}=24.9$; $Me_{CON}=32.6$; K-W=10.2, $p<0.05$; mean abundance per station: $\bar{X}_{ORG}=47.7 \pm 2.3SD$; $\bar{X}_{NT}=28.5 \pm 2.5$; $\bar{X}_{CON}=38.2 \pm 3.4$). When the pests were removed (Fig. 1f), no significant differences were found ($Me_{ORG}=12.6$; $Me_{NT}=10.5$; $Me_{CON}=11.5$; K-W=4.2, $p > 0.05$; mean abundance per station: $\bar{X}_{ORG}=13.3 \pm 0.8$; $\bar{X}_{NT}=11.2 \pm 1.4$; $\bar{X}_{CON}=15.0 \pm 1.3$).

As expected, the arthropod fauna associated with the olive canopy was more abundant in ORG, even without

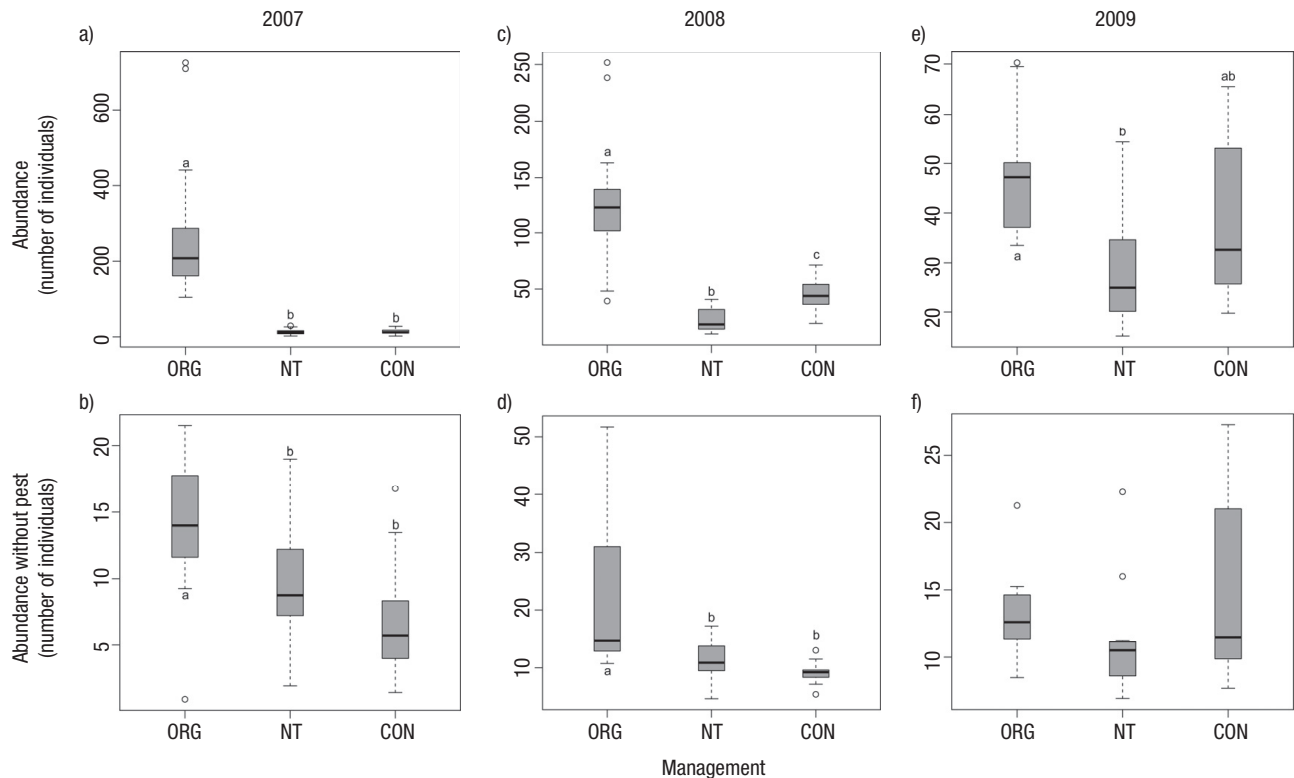


Figure 1. Median of the total arthropod abundance and abundance without pests by sampling station according to the type of management for 2007 (a and b), 2008 (c and d), and 2009 (e and f), respectively. Different letters indicate significant differences. p -value=0.05.

considering the pests, except for 2009, where no significant differences were found when pests were not considered. These results support the idea that the arthropod community is strongly influenced by agricultural practices (Santos *et al.*, 2007b). Agrochemical use and removal of vegetation cover are directly related to a decline in the abundance and diversity of beneficial arthropods (Ruano *et al.*, 2001; Cárdenas *et al.*, 2006; Porcel *et al.*, 2013). Desneux *et al.* (2007) performed an extensive review concerning the sublethal effects of pesticides, emphasizing that chemicals affect not only the mortality of beneficial arthropods but also seriously reduce their neurophysiological, physical, and behavioural capacities.

Moreover, the median and the first and third quartiles of each group per methodological sample (sampled tree) were calculated according to the management type (Table 2), as well as the mean number of individuals and its SD (Table S1 [online supplement]). Most groups proved to be most abundant in ORG compared to the two other management types. Specifically, *P. oleae*, Heteroptera, and “Other Hemiptera” showed significant differences in this sense for the three years. *E. olivina*, Formicidae, Neuroptera, and Rhaphidioptera significantly differed in two of the three years, although ORG was not always the management with the greatest

abundance. For example, Neuroptera was significantly lower in ORG for 2007 and 2009.

The pest *E. olivina* was dominant in all years, regardless of the management type, presumably due to their high biotic potential (Civantos, 1999). This psyllid seriously damages olive trees, especially increasing the percentage fall of inflorescences and reducing fruit set (Chermiti, 1992). Meanwhile, *P. oleae* sharply declined in 2008. Other studies on this lepidopteran have found considerable interannual fluctuations (Delgado & Cuesta, 1995; Hegazi *et al.*, 2011) related to environmental variables. The high importance of taxa such as Coleoptera and Araneae in the olive grove is also remarkable (Ruiz & Montiel, 2000; Ruano *et al.*, 2004; Santos *et al.*, 2007b; Scalercio *et al.*, 2009; Rei *et al.*, 2010; Cotes *et al.*, 2011), hardly register significant differences in abundance among management types. However, members of these taxa have been proposed as bioindicators of olive management at lower taxonomic levels. Cárdenas *et al.* (2006) used spider families, while Cotes *et al.* (2009) used the Coleoptera morphospecies level, both cases proving useful for differentiating between organic and non-organic management.

Moreover, taxa with significant differences for at least two of the three years were invariably flying taxa

Table 2. Median per sampling station (*Me*), first and third quartiles (*Q1* and *Q3*) of the different taxa according to the type of management (ORG: organic; NT: non-tillage; CON: conventional) and results of the Kruskal-Wallis (K-W) test applied in each case for 2007, 2008, and 2009

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	ORG (n=25)					NT (n=25)					CON (n=25)					KW-test (df=2)					ORG (n=15)					NT (n=16)					CON (n=16)					KW-test (df=2)					ORG (n=12)					NT (n=12)					CON (n=12)					KW-test (df=2)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
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- : not detected; "0.0": taxa rate <0.05. Different letters indicate significant differences. *p*-value is: *, <0.05; **, <0.01; ***, <0.005. NS: not significant.

typical of the canopy (Ruano *et al.*, 2004; Santos *et al.*, 2007b; Rei *et al.*, 2010), or with a high capacity of movement (*e.g.* Formicidae). The case of Neuroptera is particularly striking, as it was more abundant in non-organic than in ORG management (in 2008, without significant differences). Other authors have reported similar results, concluding that the greater abundance of this group in conventional olive orchard is related to a very strong dominance of *Chrysoperla carnea* (Steph.), to the detriment of other species, which on the contrary are themselves more abundant in organic management (Ruano *et al.*, 2001; Corrales & Campos, 2004; Porcel *et al.*, 2013). *C. carnea* has a high capacity to develop resistance in the treated agro-systems (Zaki *et al.*, 1999). Raphidioptera, despite its scarcity, was more abundant in ORG than in the other management types (in 2007, without significant differences). This is a rare group, whose individuals are entomophagous in all life stages; the larvae need a long development period, usually overwintering during the pupal stage (Aspöck & Aspöck, 2009). These biological

characteristics make these insects highly sensitive to any treatment or alteration of the environment, and therefore ORG management provides them a more suitable habitat for survival.

Finally, the number of taxa was found to be higher in ORG than in the other management types, nor was any group found to be exclusive to any particular type of management. This result indicates that organic management is more stable than the rest, since over three years the same taxa were recorded, whereas in other management types, they fluctuated more sharply.

Furthermore, the median and the first and third quartiles of each taxon per methodological unit based on an organic/non-organic approach (Table 3) were calculated, as well as the mean and its SD (Table S2), as confirmed by the results. In this case, only Heteroptera, Formicidae, and Raphidioptera showed significant differences for three years with respect to medians, being more abundant in organic than non-organic management.

Table 3. Median per sampling station (*Me*), first and third quartiles (*Q1* and *Q3*) of the different taxa according to the “organic/non-organic” criterion and results of the Mann-Whitney U (M-W U) test applied in each case for 2007, 2008, and 2009

Taxa	2007								2008								2009							
	ORG (n=25)			NonORG (n=50)			M-W U		ORG (n=15)			NonORG (n=32)			M-W U		ORG (n=12)			NonORG (n=24)			M-W U	
	Q1	Me	Q3	Q1	Me	Q3	U	p	Q1	Me	Q3	Q1	Me	Q3	U	p	Q1	Me	Q3	Q1	Me	Q3	U	p
ARANEAE	0.5	1.5	3.0	0.8	1.0	1.5	NS		0.5	1.5	2.0	0.6	1.0	1.9	NS		0.8	1.3	1.4	0.8	1.0	1.5	NS	
COLEOPTERA	0.6	1.0	1.6	0.0	0.5	1.3	381.5	**	0.0	0.3	1.0	0.0	0.3	0.7	NS		0.0	0.3	0.7	0.0	0.5	0.8	NS	
DERMAPTERA	0.0	0.0	0.0	0.0	0.0	0.0	NS		0.0	0.0	0.0	0.0	0.0	0.0	NS		0.0	0.0	0.0	0.0	0.0	0.2	NS	
DIPTERA	0.0	0.3	0.8	0.0	0.3	0.5	NS		0.0	0.8	3.0	0.6	1.4	2.2	NS		0.5	0.9	1.3	1.3	1.9	2.7	213.5	*
DYCTIOPTERA	0.0	0.0	0.0	0.0	0.0	0.0	NS		0.0	0.0	0.3	0.0	0.0	0.0	167.5	***	0.0	0.0	0.0	0.0	0.0	0.0	NS	
HEMIPTERA																								
<i>Euphyllura olivina</i>	136.5	188.0	276.1	0.5	3.9	6.8	0.0	***	66.0	95.3	130.3	8.3	23.3	36.0	25.0	***	17.4	21.3	36.8	11.8	15.9	26.4	NS	
Heteroptera	2.6	6.0	7.8	0.0	0.4	0.8	19.0	***	2.5	5.5	9.0	0.5	1.3	2.5	52.0	***	1.8	2.3	4.8	0.3	1.0	1.9	49.5	***
“Other Hemiptera”	0.5	1.3	3.1	0.7	2.6	5.8	NS		1.8	3.5	7.7	0.6	1.3	3.4	116.0	***	1.6	2.4	3.4	0.3	0.6	0.9	29.0	***
HYMENOPTERA																								
Formicidae	0.0	0.3	1.0	0.0	0.0	0.0	378.0	***	0.0	0.8	8.3	0.0	0.0	0.0	81.0	***	0.8	1.8	2.6	0.0	0.0	0.0	4.5	***
Hymenoptera	0.8	1.3	2.0	0.3	0.5	1.1	351.5	***	0.5	1.0	2.5	0.8	1.3	2.0	NS		1.0	1.4	2.6	1.0	1.6	2.7	NS	
LEPIDOPTERA																								
<i>Prays oleae</i>	0.3	1.5	3.5	0.0	0.3	0.8	328.5	***	0.0	0.0	0.3	0.0	0.0	0.2	NS		1.1	2.0	14.6	0.0	0.3	0.5	8.5	***
Lepidoptera	0.0	0.0	0.0	-	-	-	575.0	*	0.0	0.0	0.0	0.0	0.0	0.2	NS		0.0	0.1	0.3	0.0	0.3	0.3	NS	
NEUROPTERA	0.0	0.3	0.5	0.3	0.5	1.3	876.0	***	0.0	0.3	0.8	0.3	0.9	1.7	NS		0.3	0.4	0.8	1.8	2.3	3.0	276.0	***
ORTHOPTERA	0.0	0.0	0.3	0.0	0.0	0.0	461.5	***	0.0	0.0	0.0	0.0	0.0	0.0	NS		-	-	-	0.0	0.0	0.0	NS	
PSOCOPTERA	0.0	0.0	0.0	0.0	0.0	0.0	NS		0.0	0.0	0.0	0.0	0.0	0.0	NS		0.0	0.0	0.0	0.0	0.0	0.0	NS	
RHAPHIDOPTERA	0.0	0.0	0.1	0.0	0.0	0.0	510.0	*	0.0	0.0	0.3	0.0	0.0	0.0	157.0	***	0.0	0.1	0.5	-	-	-	72.0	*
TYSANOPTERA	0.0	0.0	0.0	0.0	0.0	0.3	789.5	*	0.0	0.3	0.7	0.0	0.3	0.5	NS		0.1	0.3	0.7	0.0	0.8	1.9	NS	

-: not detected; “0.0”: taxa rate <0.05. *p*-value is: *, <0.05; **, <0.01; ***, <0.005. NS: not significant

Accumulation curves and evaluation of the sampling intensity

By constructing accumulation curves, we evaluated the sampling effort for each year (Fig. 2a-c). In all cases, the resulting slope was $m < 0.1$ (Table 4). Furthermore, the total number of orders (TNO) which predicts the Clench adjustment proved to be very close to the observed number of orders (S_{obs}) so that the percentage of individuals recorded (FR%) was very high. In addition, the number of samples required for a slope of 0.1 ($n_{(m=0.1)}$) was in any case far below the actual number of sample units used (n). The R^2 value (parameter directly proportional to the goodness of fit) was $R^2 > 96\%$ for the three years.

Species-accumulation curves constitute a useful tool in biodiversity studies (Moreno & Halfpeter, 2000; Willott, 2001) since they i) give reliability to biological inventories and make them comparable, ii) estimate the sampling effort required for a reliable inventory, and iii) extrapolate the number of species in an inventory to estimate the total number of species in an area (Soberón & Llorente, 1993; Colwell & Coddington, 1994; Gotelli & Colwell, 2001; Jiménez & Hortal, 2003). In our case, the primary purpose of constructing accumulation curves was to evaluate the sampling intensity. All other considerations must be taken with caution, as the methodology is designed to use the taxonomic level of species rather than order (Jiménez & Hortal, 2003).

The results for 2007 helped reduce the number of sample units of 75 to 48 for the 2008 campaign, as the number of samples needed for a slope of 0.1 was found to be in any case much lower than that used. Something similar happened in 2008, thus reducing the number of sample units to 36 for 2009. Despite the steady decline in the number of samples, sampling intensity applied in the study was adequate, since the slope of the curve

Table 4. Parameters of the accumulation curves and estimators related according to the Clench fits for 2007, 2008, and 2009

Parameters	2007 (n=75)	2008 (n=47)	2009 (n=36)
a	7.2	12.6	14.7
b	0.5	0.9	1.0
m	0.00	0.00	0.01
R^2 (%)	96.2	98.9	98.2
S_{obs}	14	14	14
TNO	14.3	14.3	14.3
$n_{(m=0.1)}$	15	12	11
FR%	97.7	97.7	94.8

n =number of sampling stations (sampling unit). a and b are parameters of the adjustment curve. m =slope of the curve. If it is < 0.10 , it is considered to have reached complete inventory. R^2 =estimator of the quality of the fit. S_{obs} =number of orders found. TNO=total number of species (orders) that predict the model. $n_{(m=0.1)}$ =number of sampling units with which a value of $m=0.1$ is reached. FR% (percentage of fauna recorded) $= (S_{\text{obs}} / \text{TNO}) * 100$.

fit was in all cases above 0.1 and the percentage of fauna recorded reached very high values. Nevertheless, the number of significant differences between taxa detected in 2009 was the lowest of the three years, so that we do not recommend reducing the number of samples. In light of these results, the inventory of arthropod fauna at the level of order can be considered quite complete.

Discriminant analyses

Discriminant analysis was performed with LDA, using the 2007 data to classify the 2008 and 2009 separately, and 2008 to classify 2009. Subsequently, the weighted average percentage of success of these three tests was calculated. Two different data sets were used. In the first (analysis 1) we used all taxa while in

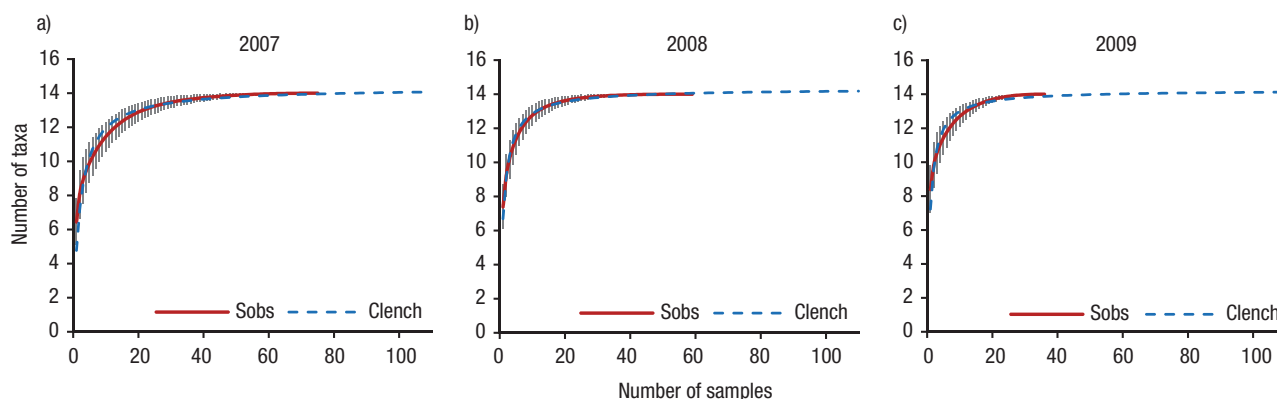


Figure 2. Accumulation curves for the orders collected in 2007 (a), 2008 (b), and 2009 (c) adjusted by Clench's equation. S_{obs} =number of order found.

the second (analysis 2) we considered only those groups with significant differences in the three years of sampling with respect to medians (Tables 2 and 3), at least under one of the two criteria (“management type” or “organic/non-organic”), regardless of the pest: Heteroptera, “Other Hemiptera”, Formicidae, and Rhaphidioptera.

Firstly, we assessed the ability of the discriminant analysis to differentiate between “management types” (Fig. 3a). The weighted average of success was 54.6% and 57.1% in analyses 1 and 2, respectively. However, when we considered the criterion “organic/non-organic” (Fig. 3b), the weighted average percentage of success increased to 86.6% (analysis 1) and 84.9% (analysis 2).

In light of these results, it may be desirable to use some other method to improve the classification rates found with the LDA method. In recent studies (Xiong & Cherkassky, 2005; Mazanec *et al.*, 2008; Moreno & Melo, 2011), one of the classification methods proposed is the SVM, the key feature of which is that it does not require prior distributional assumptions of the data because it is a nonparametric method. A third method of classification is the MDA, which can be considered an extension of the LDA, in which mixtures of normal distributions are used to make a density estimation of each class.

However, in the case of MDA, the success rates were reduced in both “management type” (Fig. 3c) and “organic/non-organic” (Fig. 3d). Only one analysis of this test was able to improve the weighted-average percentage of accuracy to 87.7%. SVM achieved higher success rates in the two analyses, both “management type” (Fig. 3e) and “organic/non-organic” (Fig. 3f), but analysis 1 failed to improve the results. In general, none of the analyses proved satisfactory, due to the high number of failures recorded, especially in classifications of 2009.

Due to interannual differences registered and disparity of classification between years, we used agroclimatic data records RIA and RAIF to characterize the different sampling dates in search of meteorological analogies that would allow us to select the most similar ones. Two distinct groups resulted (Fig. 4): one joined the first sampling dates of each year (“Group A”) characterized by having a high average humidity, sparse and scattered precipitation, and moderate average temperature and radiation (Fig. S1 [online supplement]). On the other hand, another group was formed by last dates of each year (“Group B”), *i.e.* days with low relative humidity, scarce or null precipitation, high radiation, and higher temperatures. Given the strong differences detected, we repeated the discriminant analysis with these two groups of dates separately.

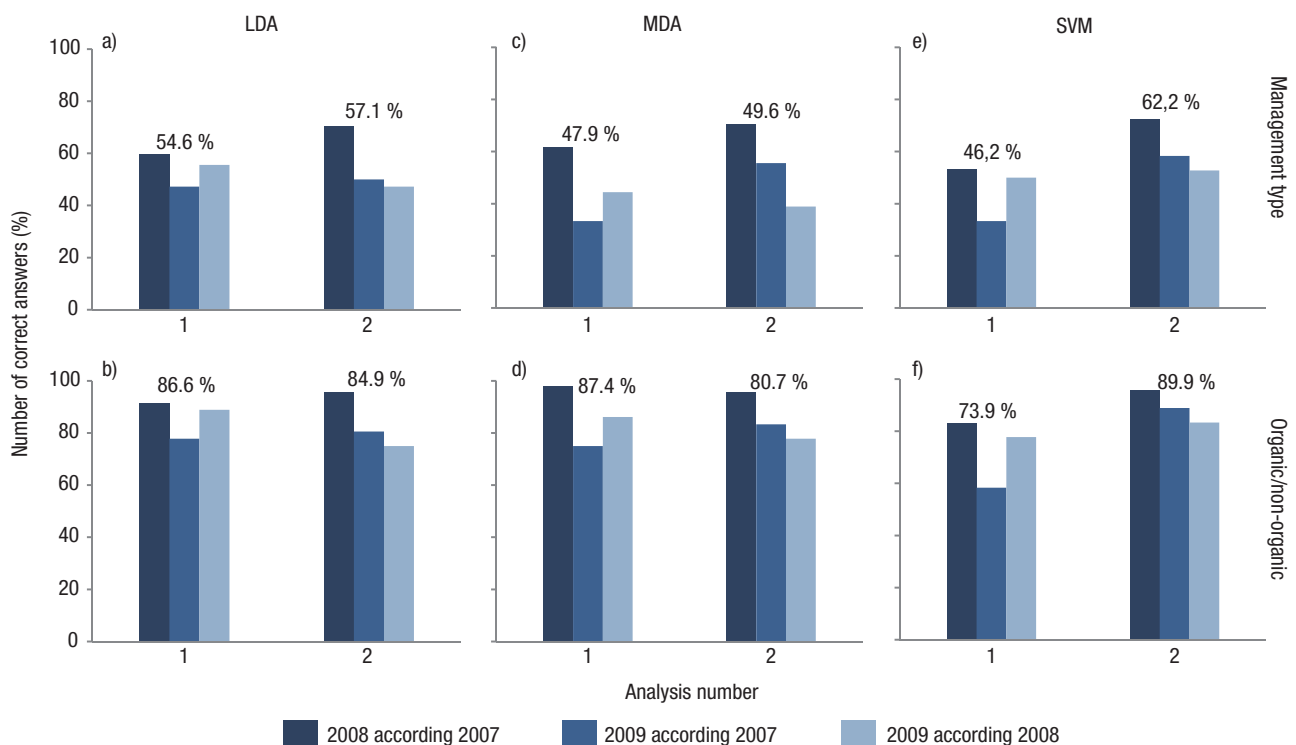


Figure 3. Discriminant analysis results with the entire group of samples using LDA (Linear Discriminant Analysis), MDA (Multiple Discriminant Analysis) and SVM (Support Vector Machine) methods. On each set the weighted mean of the three bars is shown.

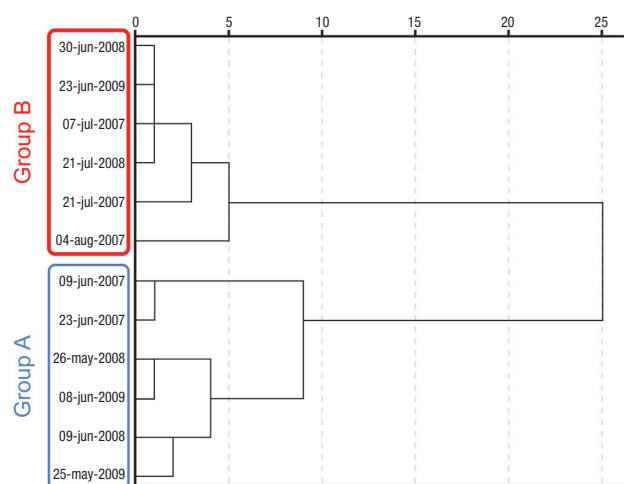


Figure 4. Dendrogram of the cluster analysis made for the meteorological data for the three sampling years.

“Group A” (Fig. 5) recorded very low average success rates for all analysis-method combinations, in “management types” (Fig. 5a,c,e) as well as in “organic/non-organic” (Fig. 5b,d,f). The number of successes in 2009 continued to be lower than in 2008.

By contrast, “Group B” (Fig. 6) improved the classification rates, especially with analysis 2. In “management types” (Fig. 6a,c,e), all three methods improved the success rate with analysis 2. It is notable that the

percentages of success in 2009 were very similar to those of 2008. Using “organic/non-organic” (Fig. 6b,d,f), we found very accurate results with analysis 2 for LDA (97.9% accuracy) and MDA (93.8% accuracy), both cases reaching 100% success in 2009 with respect to 2007 and to 2008. This result provides analysis consistency and reliability.

Discriminant analysis according to “organic/non-organic” proved to be far more effective than “management type”. In fact, in the latter, no satisfactory results were found for any of the analyses presented. Similar results have been reported by other researchers (Ruano *et al.*, 2004; Santos *et al.*, 2007b; Cotes *et al.*, 2009), perhaps due to ecological practices involving a leap in quality compared to the conservation of the ecosystem in all its aspects (Parra *et al.*, 2007). In fact, non-organic management types are more heterogeneous with respect to each other, depending on the judgement of each farmer.

The excellent results of “Group B” could be due to the meteorological homogeneity of the summer period, when weather conditions are more stable between different years. The spring term in the Mediterranean area in general is characterized by high atmospheric instability, causing numerous storm events, so that the community of arthropods from one year to another can vary greatly over this season. Cotes *et al.* (2011) found similar results, associating the improvement in clas-

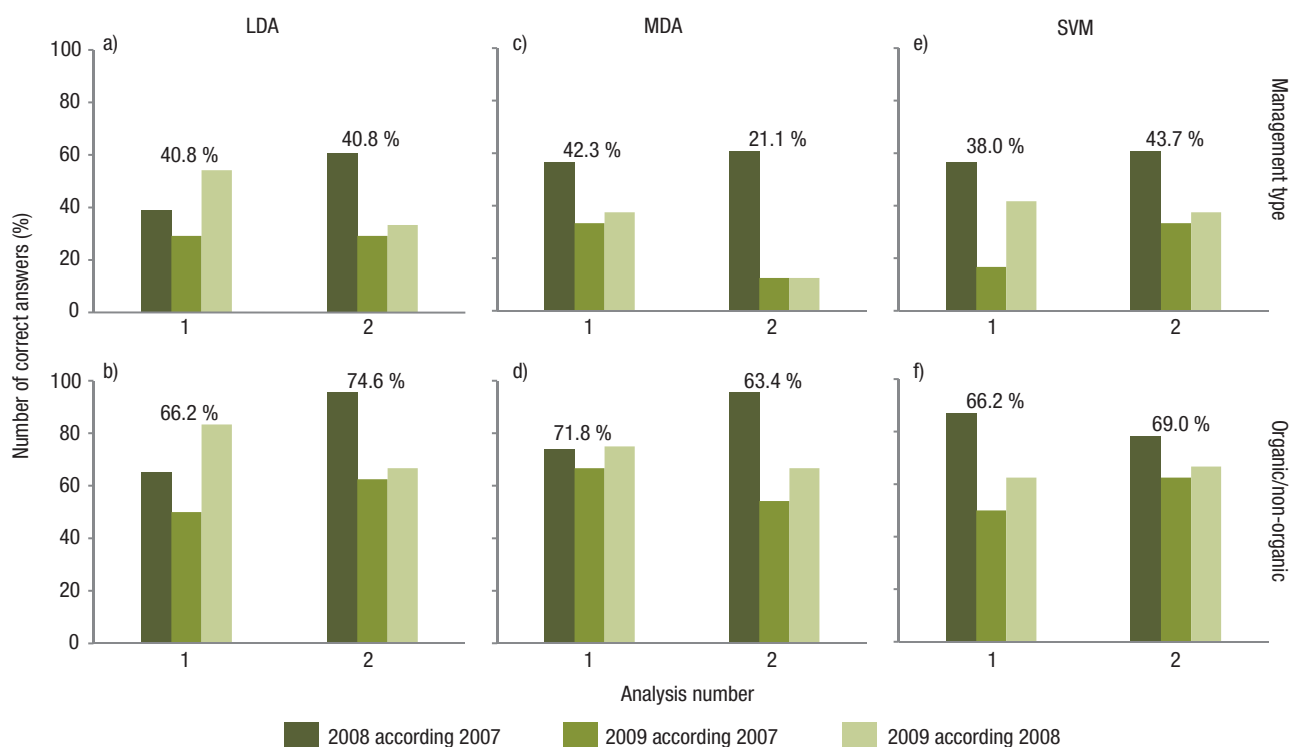


Figure 5. Discriminant analysis results with “Group A” of samples using LDA (Linear Discriminant Analysis), MDA (Multiple Discriminant Analysis) and SVM (Support Vector Machine) methods. On each set the weighted mean of the three bars is shown.

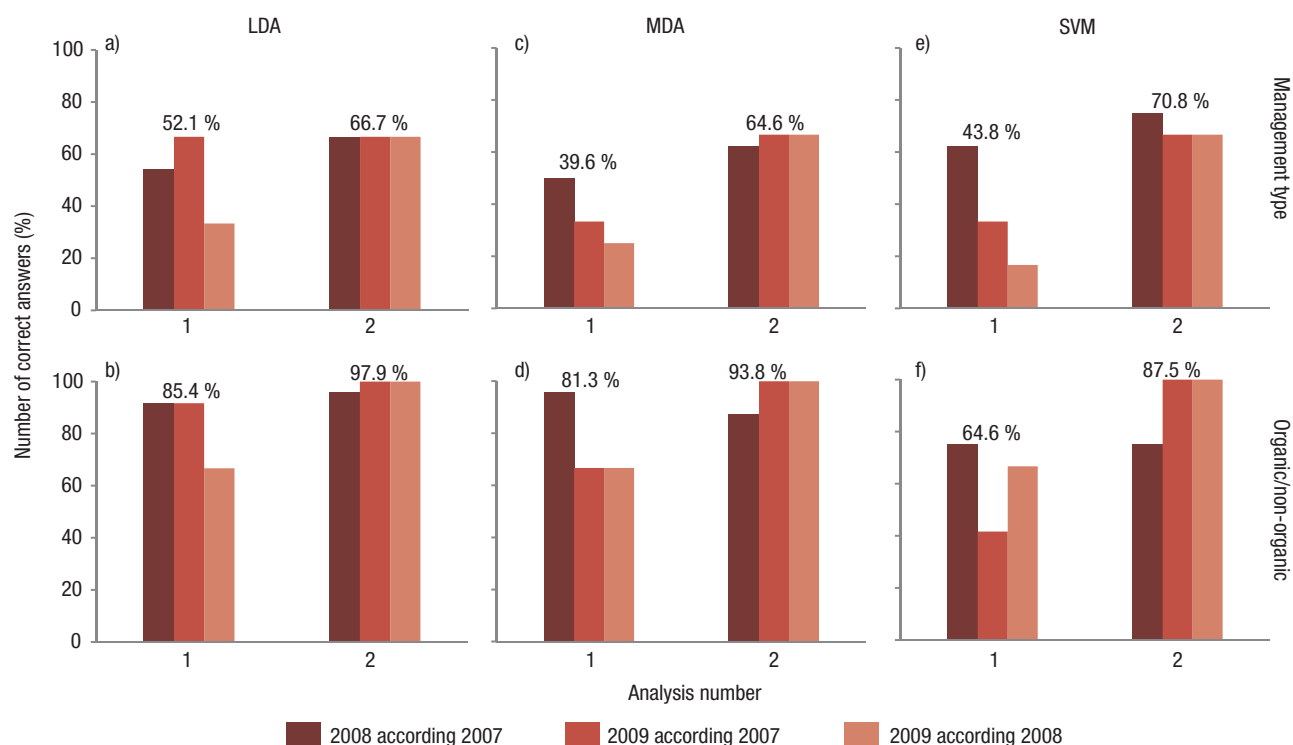


Figure 6. Discriminant analysis results with “Group B” of samples using LDA (Linear Discriminant Analysis), MDA (Multiple Discriminant Analysis) and SVM (Support Vector Machine) methods. On each set the weighted mean of the three bars is shown.

sification with the pre-blooming and post-blooming period of the olive tree.

For this reason, for discrimination between management types and especially between organic and non-organic practices, we recommend the use of taxa that differ significantly in their abundance, excluding pests. In addition, we propose beginning sampling in the second half of June and continuing it to the end of July, attempting to sample in weeks with relatively stable and homogeneous weather conditions. Moreover, the inclusion of different discriminant methods did not quantitatively or qualitatively improve the classification, so that we propose that more attention should be placed on the sampling characteristics and the data set than on the statistical method.

The use of olive-canopy arthropods at the level of order proved to be useful in discriminating between management types, especially among organic and non-organic practices. The weather should be taken into account, as the selection of the most homogeneous dates is crucial in order to quantitatively and qualitatively improve success rates. However, the inclusion of different discriminant methods did not provide significant improvement. The consideration of taxa with significant differences for the three years gave stability and reliability to the study, despite the differences between years. Therefore, for discrimination between management types, we recommend sampling between

the second half of June and late July to prevent unstable conditions in late spring and outliers in August. In addition, we propose using the discriminant analysis only for groups with significant differences, regardless of the statistical method.

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